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A review of NAFO 3LMN roughhead grenadier (*Macrourus berglax* Lacepède, 1801) reproductive biology including the evaluation of maturity ogive estimates

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ABSTRACT

A collection of 4770 histological slides collected in NAFO divisions 3LMN in a depth range from 200 to 1500 m and covering a time period between 2001 and 2015, has been reviewed to obtain information on reproductive biology of *Macrourus berglax.* Especial attention has been paid in atresia because its potential impact on stock productivity. Spatial and temporal variability of size and age at maturity of female roughhead grenadier was analyzed in NAFO divisions 3LMN based on microscopic maturity staging and individual age readings. Spawning capable females are homogeneously distributed nearly year-round, but in scarce numbers what prevent to define a spawning season. This statement united to the high levels of atresia could indicate a reproductive migration. We observed a clear decrease in length at first maturity (L_{50}) of females from 27.8 cm in the period 2005-2011 to 25.6 cm in the last four years. The age at first maturity, $A_{50,}$ varied between 13.1 and 15 years, and there is not an evident trend of change over the years.

INTRODUCTION

Roughhead grenadier (*Macrourus berglax*) is a deep-sea gadoid fish species, belonging to the Family Macrouridae, commonly referred as grenadiers. It is distributed widespread in the North Atlantic and usually found both on the shelf and the continental slope waters (Scott and Scott, 1988). The highest densities of this specie occur from 800 to 1,500 m, although it usually inhabits at depths between 200 and 2,200 m (Marshall, 1965, De Cárdenas et al., 1996; Fossen et al., 2003; Murua and De Cárdenas, 2005; González-Troncoso et al., 2012; Román et al., 2012; Vázquez, 2012). Despite that common distribution range, roughhead grenadier has been reported at depths up to 2,700 m (Wheeler, 1969). It occurs in relatively cold waters, with temperatures ranging from -0.5 to 5.4ºC (Atkinson and Power, 1987).

In Northwest Atlantic, roughhead grenadier is caught by commercial fleet but mostly as bycatch. The largest proportion is caught in Div. 3LMN in NAFO Regulatory Area, mainly in the bottom trawl fishery targeted Greenland halibut (*Reinhardtius hippoglossoides*) that is primarily performed by Spanish and Portuguese fleet (González-Costas, 2010; 2012; González-Costas et al., 2012). Roughhead grenadier catches increased until the late 90s, when catches started to decline (González-Costas, 2010).

Roughhead grenadier biological studies (stock demography, bathymetric distribution, growth, reproductive ecology and feeding habits) carried out in the Northwest Atlantic area, particularly in the Flemish Cap and Flemish Pass, are based on Russian and EU-Spanish surveys, more concretely, on the annual EU-Spanish random-stratified bottom trawl survey delivered in Flemish Cap (NAFO division 3M) since 1988 until today. In addition, Spanish sampling program for commercial fleet monitoring has yielded important information about this specie (González-Costas et al., 2012; Vázquez, 2012).

The maximum reported ages of roughhead grenadier in the NAFO areas 3LM are 20 and 28 years old for males and females, respectively, that correspond to 24 and 47 cm of maximum pre-anal fin length. Spawning activity in the North Atlantic has been reported throughout the year for this specie, with a spawning peak during winter-spring (Savvatimsky, 1989, Magnússon and Magnússon, 1995). It shows group-synchronous oocyte development (Elliassen and Falk-Petersen, 1985; Murua and Motos, 1997a; Murua and Motos, 2000) and determinate fecundity (Murua and Motos, 1996; Murua and Motos, 1997a) but maturation of oocytes seems to take more than one year (Eliassen and Petersen, 1985). The presence of atretic oocytes is a common feature in roughhead grenadier ovaries (Murua and Motos, 2000). The atresia, defined as the degeneration of developing oocytes until their complete resorption, is one of the mechanisms fish use to adjust fecundity.Atresia can occur at high levels of intensity and prevalence even limiting reproductive capacity before the spawning season begins, as has been reported in Greenland halibut (Tuene et al., 2002; Saborido-Rey et al., 2011) or redfish (Saborido et al., 2015). Roughhead grenadier total fecundity varied from 8,522 and 61,844 oocytes (Murua, 2003; Fossen et al., 2003).

The pre-anal fin length at first maturity ($L_{PA\ 50}$) for females in NAFO area is 28.5 cm whilst the age at 50% maturity was 15-16 years (Murua, 2003). Length and age at first maturity are key life history parameters for estimating spawning stock biomass and reproductive potential of fish stocks. Examination of the spatial differences in life-history parameters can be also used to better ascertain potential stock boundaries (Pawson and Ellis, 2005), and knowledge of temporal changes in such parameters offers information on stock adaption and evolution induced by external forces like environmental changes or fishing pressure, i.e. provides some clues about the stocks' resilience.

Evidence that life-history traits change in exploited populations has accumulated for decades (Miller, 1957; Borisov, 1979; Law 2000) being the most sensitive life-history trait the age at maturity, which tends to decrease in exploited populations (Trippel, 1995; Rochet, 2000). Decrease of maturity-at-length has also been observed, although in a less consistent way (Rochet, 2009).

In this study, a large collection of histological slides collected throughout 3LMN NAFO divisions in a depth range from 200 to 1,500 m covering a 11 year period (2005-2015) was reviewed to obtain information on reproductive biology of *M. berglax*. Especial attention was paid atresia because its potential impact on stock productivity.

The main objective of this work was to analyze the spatial and temporal variability of size and age at maturity of roughhead grenadier females in NAFO divisions 3LMN based on microscopic maturity staging and individual age readings.

MATERIAL AND METHODS

Histological slides collection

A total of 4,770 histological slides of *Macrourus berglax* ovaries from NAFO divisions 3LMN collected between 2001 and 2015 (**Table 1**) were available for this study. A total of 3,148 ovaries were taken in summer research surveys conducted in NAFO divisions 3M ('EU bottom trawl survey of Flemish Cap' during 2005-2015) and 'EU 3L Groundfish survey' during 2004, 2011, 2013-2015). A total of 1,622 ovaries comes from commercial Spanish bottom trawl vessels fishing in divisions 3MLN all year around during 2001, 2009-2015. Biological data of the fish were taken onboard from all individuals: pre-anal length (to the nearest 0.5 cm), weight (g) and macroscopic maturity stage according to a 4 stages protocol (immature, maturing, spawning and post-spawning). For 1,262 of the *M. berglax* females sampled in the EU bottom trawl survey of Flemish Cap, individual age readings were also available (**Table 2**).

Ovary data

Ovary samples preserved in 4% buffered formaldehyde were dehydrated, embedded in paraffin, sectioned at 3 μm and stained with haematoxylin and eosin. Histological sections were examined by light microscopy and maturity staged following the criteria of Murua and Motos (2000). The presence/absence of different oocyte developmental stages was recorded for each ovary that was classified according to the most advanced observed oocyte stage. Postovulatory follicle stage (POFs) and atresia presence/absence were also recorded.

Intensity of atresia was based on a qualitative assessment based in the technician experience; if by eye most of the leading oocyte cohort were atretic, ovaries were classified in high atresia level. Atretic oocytes are differentiated from the healthy ones because of breaks in the cellular wall (chorion), deterioration of cell nucleus, cytoplasm disorganization and vacuolization, oocyte irregular shape and a variable degree of cellular structures and components resorption.

During ovaries histological examination, different degree of parasitization was observed, so the presence of readily visible parasites and/or parasite traces in the gonads was also recorded because of the potential damage they can produce (**Fig.** 1). Females in late maturity stages (VIT3, OG, MN and H) were removed from the calculation of parasite incidence because the number of females at those stages is excessively low.

For length at maturity calculations, ovaries were classified as immature and mature. Immature ovaries were those presenting all oocytes in primary growth stage and mature ovaries those presenting the rest of oocyte developmental stages (cortical alveoli, vitellogenic and atretic oocytes) or any sign of previous spawning activity (post-ovulatory follicles).

Evaluation of maturity ogives

First of all, an exploratory analysis of samples from commercial and oceanographic surveys was carried out to identify potential sources of bias derived from the different sources of information.

The exploratory analysis revealed that samples from years 2001 and 2004 had very low levels of sampling and they were removed from the analysis. As commercial sampling is not regularly distributed between months and years, and in order to avoid bias only research survey samples (3M and 3L divisions) were used for evaluation in size at maturity estimates.

Age at maturity was evaluated only in samples from the EU bottom trawl survey of Flemish Cap with available age readings.

Generalized linear models (GLMs) were used to investigate the proportion of mature females at length and to statistically examine spatial and temporal differences in L_{50}**.** Presence of parasite was introduced in the model

because it could cause maturity alteration. Maturity state was treated as a binomial response variable with logit link function and modelled as a function of length, with NAFO division, year and the presence of parasite as covariates. Year, division and presence of parasite were modelled as categorical variables and pre-anal fish length as a quantitative variable. Significant main effects and interactions were included in the GLM using a forward and backward stepwise selection procedure. We tested the significance of the GLM terms by using the chi-square (χ^2) approximation test. Size of maturity was defined by the body length at which 50% of the population was mature (L_{50}) and was estimated from the GLM predictions.

The confidence intervals at 95% for the L_{50} and predicted maturity ogives were estimated by non parametric bootstrap of the observations using 1000 replicates.

Significant ($p < 0.05$) pairwise differences were identified by using Tukey's HSD post hoc tests.

As all individuals with age readings belonged to division 3M, temporal differences in age at maturity were studied exclusively in this area.Maturity ogives and A_{50} were estimated by year with GLMs and likelihood ratio tests were performed to determine whether the shapes of the maturity curves were statistically different.

All model fits and posterior estimates were conducted in the statistical environment R version 3.2.5 (R Development Core Team, 2016).

RESULTS

Stages of oocyte development

Table 3 shows the numbers of immature and mature females presenting different stages of oocyte development (the most advanced stage in the ovary) in terms of length interval. From a total of 4,770 females, 2,543 (53,3%) were immature, 2,227 (46,7%) mature and only 129 (2.7%) were at spawning or spawning capable (VIT3, OG, MN or H) stages.
Table 4 shows the number of individuals from 3M division from which individual age readings are available.

Annual cycle of gonad development

Most samples (73%) were collected in summer time (June to August), when the research surveys are carried out and samples from commercial vessels cover the rest of the year. The number of commercial samples by month ranges between 25 ovaries in November to 289 in April (**Table 5**). Samples are available from all seasons, but spawning capable ovaries are very scarce, with top rates in July (3%) and August (3,8%), likely because research sampling is more intensive and cover areas with no interest to fishing vessels. Summarizing, spawning activity is distributed all around the year but is very low. Thus no spawning period can be described.

Atresia

Table 6 shows the prevalence of females with high level of atresia by NAFO division and year in samples taken during the summer research surveys. Prevalence increases from a 24% in ovaries at Late Cortical Alveoli stage to a 100% in vitellogenic ovaries. Prevalence of vitellogenic females with high level of atresia is always higher than 60%, except in 2009 (57%) and in 2010 (33%), going over the 90% the last five years in both divisions except the year 2014 in 3M (63%).

Variation of atresia prevalence along the year was studied by including commercial samples information (**Table 7**). Most of the commercial sampling was carried out in 3L division, and that's why samples from the

whole year are available, although samples from June and September are very scarce. In this division, from May to September, the prevalence of high atresia reaches 100%, except in August, when prevalence overcome the 85%. In 3M division, from May to September prevalence is also high, reaching the 100% in May and July but the number of vitellogenic ovaries in May is very low. Commercial sampling is very limited in 3N. Despite the low number of samples, values in 3N are in accordance with 3L in those months with enough samples, *i. e.*, April (57%), July (100%), and October (50%).

Parasites

A 22 % of ovaries were seriously damaged, presenting parasites or traces of parasite presence (**Fig.** 1). Thus, in 3L division percentage of parasite occurrence decreases progressively from a 36% in 2009 to a 10% in 2015. 3M division series let us distinguish alternating periods of higher and lower parasite incidence, being the higher occurrence in the period 2007-2011. However, in 3M division some years breaks the trend, bringing us to the need of deeper studies.

Spatial and temporal differences in length at maturity

According to GLMs results, the proportion of mature females at length has varied significantly through the years. Specifically, females from 2012 to 2015 were mature at significantly smaller lengths than females during 2005-2011 (25.60 and 27.78 cm respectively).

A full model, including length and year (with levels: 2005-2015) and division (levels: 3L and 3M) as covariates, was built to evaluate spatial and temporal changes in maturity. Both, the division and the interaction year:division were removed from the final model due to the low significance level, the low variance explained and the small difference in the AIC value. Then, the final model only included the main effects of length and year (**Table 8**). No differences in maturity schedules between divisions 3L and 3M were detected by the model.

Performed likelihood ratio tests determined that the maturity at length schedule for years 2006 - 2011 was no significantly different ($p > 0.05$) from the base year (2005) but significant differences in the intercept were found for years 2012 to 2015 ($p < 0.0005$) (Table 8, Figure 2). L_{50} ranged from 28.5 cm in 2006 to 25.1 cm in 2015 . Since 2009 a steady decreasing trend in size of maturity is observed (**Table 9**, **Fig**).

Pair comparison analysis shown that L_{50} differed significantly between years; 2013, 2014 and 2015 were significantly different from years 2005 to 2011. 2012 is different from 2005, 2006, 2007 and 2009 (**Fig**). Two time-periods were set: 2005-2011 and 2012-2015. A GLM model with length as continuous variable and time-period as factor was fit. The GLM results confirmed the differences in maturity intercept between the two time-periods ($p < 0.001$) (**Table 10**). A single maturity ogive by time-period was estimated by pooling samples across years; the estimated L_{50} for years 2005 to 2011 was 27.8 cm and 25.6 cm for the period 2012-15.

We explored the possibility that the observed differences in maturity ogive and L_{50} could be attributed to the presence of parasites in the ovary. To evaluate this effect, the parasite presence was included as a predictor in a GLM model with length and time-period as other covariates. Through the stepwise procedure the variable parasite was excluded from the final model that included length and period as significant explanatory variables and a no significant interaction Length:Time-period (**Table 11**). These results suggest that maturation is not affected by parasitic infection.

Temporal differences in age at maturity in 3M division

Using generalized linear models, there seem to be a slight general decline of age at maturity from 2005 to 2014 (**Fig.4**) but fluctuations break this tendency, especially in 2009 when age at maturity slightly exceeds

2005 value. *Post hoc test* results indicate that years 2013 and 2014 was significantly different from 2005, 2009 and 2011 and that A_{50} in year 2012 was significantly different from 2005 and 2009 (**Fig.4**).

Fig. 5 shows the maturity ogives and A_{50} by year. The A_{50} for females of roughhead grenadier varied from a maximum value of 15 years in 2009 to a minimum value of 13.1 in 2013. The A_{50} value for the whole period was 13.98 years. Maturity ogives at age of females for the period 2005-2014 based on GLM results are given in **Table 12**.

DISCUSSION

Roughhead grenadier spawning season

In the Labrador Sea, a well defined spawning season from the end of spring to the beginning of summer was reported by Geistdoerfer (1979). On the Grand Bank, Savvatimsky (1984) described a spawning season in winter and early spring. More recent publications on NAFO divisions 3LMN (Murua and Motos, 2000) inferred a spawning period from February to July, although other authors warn of the poor information. In this study, additional collection during all months of the year didn't add more information to help characterize the spawning season for this species because the number of spawning and spawning-capable females was very scarce. We found females with ripe oocytes nearly year-round but in scarce numbers and a peak of spawning could not be identified in this population.

Atresia and possibility of an adolescent phase

We found that prevalence of roughhead grenadier females with high levels of atresia in early vitellogenesis is very high. Although the intensity of atresia was qualitative assessed and based on technician experience, they are highly experienced in microscopic interpretation of ovary maturity and atretic states of other species in the area. We are working also with Greenland halibut from the same NAFO area and a similar pattern of atresia is observed in the early vitellogenesis stages. In fact, this high atresia in Greenland halibut was previously studied during the bilateral project between Canada and Spain STREPHALIBUT, in 2009-2011 (Saborido et al., 20111), suggesting that atresia in oocytes during the early stages of maturation (cortical alveoli and initial vitellogenesis) could indicate an early fertility regulation. However, a quantification of atresia levels should be done.

It seems that there are more similarities between Greenland halibut and roughhead grenadier reproductive strategies. There are suggestions that Greenland halibut are not capable of spawn every year (Junquera et al., 2003) and it has been stated that oocyte maturation takes more than one year (Kennedy et al., 2011). For roughhead grenadier Eliassen and Falk-Petersen (1985) suggest that the maturation oocyte takes more than one year too.

In Greenland halibut, the ovary initially develops as far as early vitellogenesis, but it remains unclear the time scale for that (Kennedy et al. 2011). Junquera et al. (2003) classify females as 'adolescent' when they were at either the cortical alveoli stage or any level of vitelogénesis without signs of previous spawning and observed a distance of 4 years between the adolescent and spawning stages.

According to our results, roughhead grenadier could also have an 'adolescent' period. Thus, fish begin maturation by first time could undergo massive atresia and this process could be repeated more than one year before spawning for first time ("adolescence"), but this phenomenon requires further studies before reaching a robust conclusion about the adolescence of *M. berglax*. Equally, it is important to study the implications of this strategy in their reproductive potential and stock dynamic.

As stated before, spawning capable females are homogenously distributed nearly year-round but in scarce number what prevent to define a spawning season in the NAFO dividiosn 3LMN. This statement linked to the high levels of atresia (adolescence) of most of vitelogenic females in the area makes us to suspect that there

could be a reproductive migration of roughhead grenadier, being only the immature, adolscents, bad condition specimens and/or regenerating females which are available to sampling and fishing in 3LMN areas. This migration could be along the slope towards deeper waters or a migration towards a different area.

Parasites

Almost one-quarter of females of roughhead grenadier population in 3LMN was parasited or had traces of having parasites. The potential damage caused to the gonad could be high , affecting fecundity to a greater or lesser extent. Thus, these high rates of infestation should be taken into account in fecundity estimation.Diversity of parasites infecting roughhead grenadier gonads is still unknown. Previous analyses in some ovaries from the division 3L identified a digenean parasite that belongs to the species *Gonocerca macroformis*. But the identity of this species was questioned by specialized taxonomists because *G. macroformis* has some similarity to the species *Gonocerca phycidis* and identification has not improved given the limited citations since 1954 (Gibson, 1996). Palm and Klimpel (2008) reported that deep sea parasite fauna of roughhead grenadier is relatively uniform between years and showed no distinct interannual variation. But in our study we found evidence of alternating periods of higher and lower ovaric parasite incidence.

To know parasitic species infecting grenadier gonads is important because depending on the parasite life-cycle and physiology, the impact on roughhead grenadier reproduction may change. In the present study we only focused on macroscopic parasites, and no relationship to maturity was detected. However, if parasite complete their entire life cycle in *M. berglax*, it could considerably reduce the reproductive capacity of the host through direct nutrient depletion (Barber and Wright, 2005). Gonadogenesis is typically delayed, impaired, or reversed in fish that are under nutritional stress, so it could be expected that parasite infections be associated with reduced gonadogenesis or fecundity (Chen and Power, 1972; Wiklund et al., 1996). One possibility is that those highly parasitized individuals be in poor physiological condition and remains as immature or does not migrate to spawning areas. Anyway, more specific studies and comparative analyses have to be carried out to determine the impact of parasitism on *M. berglax* reproductive potential and stock dynamic in the NAFO divisions 3LMN in relation to other areas of distribution of this specie in the North Atlantic.

Evaluation of maturity ogives

In NAFO divisions 3LMNO biomass index of roughhead grenadier show a clear decreasing trend since 2009 (NAFO, 2015). Density-dependent effects may explain a decrease in length/age at first maturity in populations that have shown extensive declines in biomass (Nash et al., 2000; Engelhard and Heino, 2004). The most documented effect is a decrease in age at maturity (Trippel, 1995) but this has not been exactly the case with roughhead grenadier. Thus, although our results showed that there seem to be a slight general decline of age at maturity from 2005 to 2014, fluctuations break this tendency and there are no significative differences in A_{50}. However, results of previous studies in the area show A_{50} values consistent with the trend of biomass indices available in the adjacent divisions 3NO. Thus, in the mid nineties, A_{50} was estimated in 13.5 years (Murua and Motos, 1996) and a higher value (15.6 years) several years later in the period 1998-2000 (Murhua, 2003) matching with an increase of biomass index (NAFO, 2015). However, ring pattern growth of otoliths of roughhead grenadier is not clear, leading to some level of uncertainity in age reading what might be behind these apparent changes in age at maturity. In any case, they are not significant, so we could conclude that no differences in maturity ogive by age of *M. berglax* has been detected during the last ten years.

We found a significative decrease of length at maturity in the last four years. This decreasing in size at maturity can be related to environmental changes that diminished food availability (Silva et al., 2006), an increase of the interespecific competition or to fishing induced changes.. Fishing usually targets on large specimens, biasing the age and length composition of stocks what directly impact on reproductive potential

through a reduction of fecundity, size and viability of eggs, and spawning season duration and timing (Rochet, 2009). However, this statement has to be made with caution, as most experimental studies suggest that 10 - 20 generations are needed for selection to occur (Swain et al., 2005) and *M. berglax* is a long-live species. In addition, results of previous studies in the area report fluctuations in size at maturity values, as L_{50} increased from 26,2 cm in 1995-1996 (Murhua and Motos, 2000) to 28,5 cm in 1998-2000 (Murhua, 2003). In any case, *M. berglax* is characterized by a slow growth and late maturation (Savvatimsky, 1994). These life history characteristics made this species especially vulnerable to environmental variations and very sensitive to overfishing what could provoke a rapid decline of stocks abundance and biomass. Therefore, this species need to be managed with caution to prevent population decrease or genetic changes that could become irreversible and result in stock collapse.

Conclusions

Overall, this study shows a decreasing trend of size at maturity of *M. berglax* in NAFO divisions 3LMN, as well as a high prevalence of ovaric parasitation –what could decrease dramatically fecundity or even avoid spawning- and females at early ovary developmental stage with high levels of atresia that suggests they will undergo to a skip spawning process.

These facts joint to the absence of a relevant number of actively spawning females could indicate that the areas/depths covered in this study correspond to nursery and/or recovering areas where only adolescent, recovering individuals or skip spawners are available to our sampling. A spawning migration to adjacent waters, or to deeper watersmay occur. If this is the case, the monitoring program is not sampling a representative portion of the population and our results have be applied with caution to the rest of stock.

Further work

It is necessary to investigate atresia impact in a greater extent, i.e., intensity of atresia and high levels of atresia in relation to age -looking for the possibility of an adolescent period of one or more years- and environmental variables including parasitation and fishing pressure. Regarding reproductive ecology, we should make exploratory sampling and analyses to check if spawning capable females are present at greater depths in NAFO divisions 3LMN, as well as contrast our results with other studies about *M. Berglax* reproductive acitivity in adjacent areas to test the hypothesis of a reproductive migration..

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Table 1. Number of gonads collected and processed from Spanish commercial and research surveys in the period of study. Research surveys samples in 3M division come from 'EU bottom trawl survey of Flemish Cap' and those from 3L division are sampled during '3LNO Groundfish survey'.

	COMMERCIAL SAMPLING					RESEARCH SRUVEYS				
Year	Month	Number of gonads			Length range(cm)	Month	Number of gonads			Length range(cm)
		3L	3M	3N			3L	3M	3N	
2001	2	13			10-35					
	3	54	28		12-37					
	4	38			11-38					
	5	19			20-34					
2004						7-8	137			9-38
2005						6-7		326		20-46
2006						6-7		298		20-45
2007						6-7		304		20-43
2008						6-7		308		9-42
2009	11	5			25-36	6-7		314		13-43
	12	40			25-36					
2010	1	10			25-32					
	2	12			25-33					
	3	25	5		25-31					
	5	51	28		10-35					
	6	4			19-26	6-7		218		13-39
	8	89			16-40					
	9	46			16-35					
	10	56		56	15-37					
	11	7		13	14-36					
2011	4	80			15-38					
	5	14	15		24-39					
	7	48	16		19-41	6-7		227		18-42
	8	17	36		20-36	7-8	88			18-40
	9	10	18		20-28					
2012	1	36			17-32					
	3	9			15-29					
	4	14		70	20-36					
	5		97		20-41					
	6					6-7		138		20-40
	10			70	18-35					
	12	13			20-29					
2013	1	13		14	20-30					
	3		20		20-37					
	4	6			24-33					
	6	22			15-33	6-7		133		16-42
	8	43			19-34	7-8	142			18-40
	9	6			20-33					
2014	1	22		15	20-35					
	3	25			20-30					
	7	1	4	31	15-34	6-7		128		14-41
	8	3		19	15-29	7-8	135			20-40
2015	2	49			16-28					
	3	28	13		17-34					
	4	46	35		14-37					
	5	39	6		16-30					
	6					6-7		113		14-38
	8					7-8	139			20-39
Total by division		**1013**	**321**	**288**			**641**	**2507**	**-**	
Total Comercial surveys: 1622						**Total research surveys: 3148**				

Table 2. Number of *M. berglax* females withhistological-maturity data available by year and age. Samples were taken during the EU bottom trawl survey of Flemish Cap series.

EU bottom trawl survey of Flemish Cap											
Age	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Total**
6					2					1	3
7					2	2					4
8					4	1		1	1	1	8
9	2	1	1	3	4	8	10	4			33
10	11	2	5	7	2	16	18	12	8	6	87
11	10	7	14	6	11	20	23	12	18	17	138
12	10	6	6	5	5	18	26	22	20	16	134
13	17	4	10	9	10	20	21	18	10	19	138
14	15	5	9	5	10	30	23	16	21	12	146
15	11	9	7	8	9	12	21	14	14	13	118
16	10	8	8	3	9	22	20	3	2	9	94
17	9	7	9	9	4	7	14	11	14	8	92
18	10	8	4	7	5	10	10	4	4	13	75
19	10	9	5	5	8	5	4	4	5	1	56
20	8	8	7	3	5	2	13	2	2	2	52
21	2	1	9	2	4		4	2	2	1	27
22	4	2	1	2	4	4	2	1	3	2	25
23	2	3	1		4	3	5	1	2		21
24						1	1				2
25						2	2	1			5
26									1		1
27									1		1
29									1		1
32		1									1
Total	**131**	**81**	**96**	**74**	**102**	**183**	**217**	**128**	**129**	**121**	**1262**

Table 3. Numbers of females presenting different oocyte developmental stage (the most advance) by size interval. Spawning capable and actively spawning females are shaded in grey. CN: Chromatin nuclear; PN Perinucleolar; CA: Cortical alveoli; LCA: late cortical alveoli; VIT1: early vitellogenesis; VIT2: medium vitellogenesis; VIT3: late vitellogenesis; OG: Oil globule formation; MN: Migratory nucleus; H: Hydrated oocytes.

Pre-anal	**Immature**		**Mature**								
length(cm)	**CN**	**PN**	**CA**	**LCA**	**VIT1**	**VIT2**	**VIT3**	**OG**	**MN**	**H**	**Total**
9-11	5										5
11-13	12										12
13-15	36										36
15-17	67	1									68
17-19	99	5	1	2							107
19-21	345	37	5	1							388
21-23	446	167	23	3			1				640
23-25	288	279	92	16	2	1	2	1		1	682
25-27	126	296	175	76	18	3	3	5		1	703
27-29	30	194	256	129	45	4	6	7		2	673
29-31	3	75	225	175	56	11	10	15	1	3	574
31-33	1	23	111	170	75	7	8	15	3	3	416
33-35	1	3	46	99	51	10	7	8	3	4	232
35-37		3	19	64	35	3	4	4	1	1	134
37-39	1		5	17	32	3	1	4		1	64
39-41			1	7	14	2	2				26
41-43					6			1			7
43-45				1	1						2
45-47									1		1
Total	**1460**	**1083**	**959**	**760**	**335**	**44**	**44**	**60**	**9**	**16**	**4770**

Table 4. Numbers of females presenting different oocyte developmental stage (the most advance) by age. Spawning capable and actively spawning females are shaded in grey. CN: Chromatin nuclear; PN Perinucleolar; CA: Cortical alveoli; LCA: late cortical alveoli; VIT1: early vitellogenesis; VIT2: medium vitellogenesis; VIT3: late vitellogenesis; OG: Oil globule formation; MN: Migratory nucleus; H: Hydrated oocytes. All samples are from NAFO area 3M.

Age	Immature		Mature								
(Years)	CN	PN	CA	LCA	VIT1	VIT2	VIT3	OG	MN	H	Total
6	3										**3**
7	4										**4**
8	7	1									**8**
9	30	3									**33**
10	85	2									**87**
11	102	31	4	1							**138**
12	63	56	11	3			1				**134**
13	38	62	30	5	2		1				**138**
14	10	59	57	18	2						**146**
15	4	24	58	23	4		1	4			**118**
16	2	10	43	25	11	1	1	1			**94**
17		3	30	36	16	2	2	3			**92**
18			22	31	15		2	4		1	**75**
19		1	14	20	16		1	4			**56**
20		1	5	28	11		2	4		1	**52**
21			1	17	7	1				1	**27**
22			3	8	12	1		1			**25**
23			1	7	8	1		3	1		**21**
24				1	1						**2**
25					4		1				**5**
26								1			**1**
27					1						**1**
29								1			**1**
32			1								**1**
Total	**348**	**253**	**280**	**223**	**110**	**6**	**12**	**26**	**1**	**3**	**1262**

Table 5. Number of females at different oocyte development stage (the most advance) by month. Spawning capable and actively spawning females are shaded in grey. CN: Chromatin nuclear; PN Perinucleolar; CA: Cortical alveoli; LCA: late cortical alveoli; VIT1: early vitellogenesis; VIT2: medium vitellogenesis; VIT3: late vitellogenesis; OG: Oil globule formation; MN: Migratory nucleus; H: Hydrated oocytes.

Oocyte stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
CN	32	17	73	75	95	355	489	219	31	59	5	10	1460
PN	17	25	56	70	65	254	308	200	34	42	7	5	1083
CA	21	12	31	41	40	241	326	194	8	32	3	10	959
LAC	22	12	19	48	42	135	212	206	5	40	7	12	760
VIT1	12	5	14	36	16	51	103	71	1	8	3	15	335
VIT2	3	3	9	12	4	1	4	8					44
VIT3	1		2	6	4	12	9	10					44
OG			2			12	26	18	1	1			60
MN	1				1		3	4					9
H	1		1	1	2	1	6	3				1	16
Total	**110**	**74**	**207**	**289**	**269**	**1062**	**1486**	**933**	**80**	**182**	**25**	**53**	**4770**
% spawning capable + actively spawning	2.7	0.0	2.4	2.4	2.6	2.4	3.0	3.8	1.3	0.5	0.0	1.9	

Table 6. Prevalence of females presenting a high level of atresia by NAFO division, year and the most advanced oocyte developmental stage in the ovary. 3M samples were taken during the 'EU bottom trawl survey of Flemish Cap' and 3L samples were taken during '3LNO Groundfish survey'. The total number of sampled females (n) are also presented.

		Prevalence of females with high atresia level (n)							
Division	3L Year	AC	ACT	VIT1	VIT2	VIT3	GL	NM	H
3L	2004	3 (30)	50 (10)	75 (8)		0 (1)	0 (1)		
Jul-Aug	2011	0 (10)	48 (23)	82 (11)		100 (1)	0 (1)		
	2013	6 (35)	50 (26)	100 (9)		0 (2)	0 (3)		
	2014	3 (37)	35 (31)	90 (10)	100 (1)		25 (4)		
	2015	0 (33)	45 (47)	100 (7)		100 (1)	0 (1)		
3M	2005	0 (61)	24 (54)	85 (13)	60 (5)	40 (5)	29 (7)	0 (2)	0 (1)
Jun-Jul	2006	6 (70)	52 (23)	63 (19)	100 (1)		0 (3)	0 (1)	
	2007	5 (74)	47 (38)	92 (12)			0 (5)		0 (1)
	2008	5 (78)	43 (30)	71 (7)		0 (3)	50 (2)		
	2009	5 (65)	35 (34)	57 (23)		0 (3)	0 (2)	0 (1)	100 (1)
	2010	6 (47)	38 (21)	33 (21)		0 (4)	0 (4)		0 (1)
	2011	0 (32)	45 (53)	85 (20)	100 (1)	0 (1)	100 (1)		0 (1)
	2012	6 (31)	44 (25)	92 (13)			50 (2)		0 (1)
	2013	0 (33)	54 (24)	100(10)	100 (1)	0 (1)	14 (7)		
	2014	8 (36)	44 (25)	63 (8)		0 (3)	0 (1)		
	2015	0 (29)	64 (28)	88 (8)	100 (1)		40 (5)	100 (1)	0 (2)

Table 7. Prevalence of females presenting a high level of atresia by NAFO division (3LMN), month and the most advanced oocyte developmental stage in the ovary. Both commercial and research survey samples from NAFO area 3MLNO are included. Late cortical alveoli and vitellogenesis are shaded in grey. The total number of sampled females (n) are also presented.

		Prevalence of females with high atresia level (n)							
Division	Month	AC	ACT	VIT1	VIT2	VIT3	GL	NM	H
3L	Jan	0 (11)	21 (19)	82 (11)	50 (2)	0 (1)		0 (1)	0 (1)
	Feb	0 (12)	33 (12)	0 (5)	0 (3)				
	Mar	0 (18)	7 (14)	20 (10)	17 (6)		0 (1)		0 (1)
	Apr	0 (21)	27 (22)	59 (27)	22 (9)	0 (6)			0 (1)
	May	0 (17)	64 (22)	100 (11)	67 (3)	33 (3)			50 (2)
	Jun	0 (5)	0 (6)	100 (2)	0 (1)	100 (1)			
	Jul	2 (52)	44 (34)	100 (9)		33 (3)	0 (6)		
	Aug	3 (131)	50 (139)	89 (54)	67(3)	40 (5)	18 (11)	0 (2)	0 (2)
	Sep	14 (7)	40 (5)	100 (1)			0 (1)		
	Oct	0 (10)	17 (6)	50 (2)			0 (1)		
	Nov	0 (2)	100 (3)	67 (3)					
	Dec	0 (10)	0 (12)	20 (15)					0 (1)
3M	Mar	0 (13)	60 (5)	75 (4)	100 (3)	50 (2)	0 (1)		
	Apr	0 (7)	29 (7)	0 (2)					
	May	0 (23)	25 (20)	100 (5)	0 (1)	0 (1)		100 (1)	
	Jun	3 (236)	45 (129)	69 (49)		0 (11)	17(12)		0 (1)
	Jul	6 (268)	48 (174)	70 (93)	100 (4)	33 (6)	20 (20)	33 (3)	17 (6)
	Aug	0 (61)	25 (63)	94 (17)	60 (5)	40 (5)	29 (7)	0 (2)	0 (1)
	Sep	0 (1)							
3N	Jan	10 (10)	67 (3)	100 (1)	0 (1)				
	Apr	0 (13)	42 (19)	57 (7)	0 (3)				
	Jul	0 (6)	75 (4)	100 (1)					
	Aug	0 (2)	100(4)						
	Oct	0 (22)	21 (34)	50 (6)					
	Nov	0 (1)	50 (4)						

Table 8. Summary of GLM's results by year. Data from both divisions, 3L and 3M; are pooled together.

Source	Estimate	Std. Error	z value	P value
Intercept	-19.20213	0.71873	-26.72	< 0.0001
Length	0.68821	0.02506	27.46	< 0.0001
Year (2006)	-0.40515	0.25639	-1.58	0.11
Year (2007)	0.08962	0.25163	0.36	0.72
Year (2008)	0.23158	0.25077	0.92	0.36
Year (2009)	-0.01528	0.24920	-0.06	0.95
Year (2010)	0.25052	0.28574	0.88	0.38
Year (2011)	0.48516	0.25258	1.92	0.05
Year (2012)	1.13097	0.31511	3.59	< 0.0005
Year (2013)	1.53146	0.26335	5.82	< 0.0001
Year (2014)	1.57625	0.26735	5.90	< 0.0001
Year (2015)	1.92876	0.27656	6.97	< 0.0001

Table 9. L_{50} estimates and bootstrap uncertainty associated by year.

Year	L_{50} original	Bootstrap Std error	Bootstrap 95% Confidence interval
2005	27.90	0.228	27.44 – 28.33
2006	28.49	0.267	27.99 – 29.02
2007	27.77	0.282	27.25 – 28.35
2008	27.56	0.273	27.04 – 28.12
2009	27.92	0.261	27.42 – 28.43
2010	27.54	0.369	26.79 – 28.27
2011	27.20	0.269	26.66 – 27.71
2012	26.26	0.358	25.58 – 26.93
2013	25.68	0.275	25.13 – 26.21
2014	25.61	0.284	25.09 – 26.19
2015	25.10	0.293	24.53 – 25.67

Table 10. L_{50} estimates and bootstrap uncertainty associated by time-period.

Time-period	L_{50} original	Bootstrap Std error	Bootstrap 95% Confidence interval
2005-2011	27.78	0.107	27.58 – 27.99
2012-2015	25.60	0.152	25.29 – 25.88

Table 11. Analysis of deviance from the final GLM model for maturity at length for two time-periods and presence of parasites.

	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
NULL			3010	4167.7	
Length	1	2129.98	3009	2037.7	< 0.0001
Time-period	1	136.51	3008	1901.2	< 0.0001
Length:Time-period	1	2	3007	1899.2	0.156

Table 12. Female maturity ogive at age for roughhead grenadier based on GLM results.

	Age																							
year/age	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24+**
2005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.10	0.26	0.54	0.79	0.93	0.98	0.99	1.00	1.00	1.00	1.00	1.00
2006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.15	0.36	0.65	0.86	0.95	0.98	1.00	1.00	1.00	1.00	1.00	1.00
2007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.06	0.18	0.42	0.71	0.89	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00
2008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.12	0.31	0.60	0.83	0.94	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
2009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.23	0.49	0.76	0.91	0.97	0.99	1.00	1.00	1.00	1.00	1.00
2010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.09	0.25	0.52	0.78	0.92	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00
2011	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.06	0.18	0.41	0.70	0.88	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00
2012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.06	0.16	0.39	0.67	0.87	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.07	0.21	0.46	0.73	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.07	0.19	0.43	0.71	0.89	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00

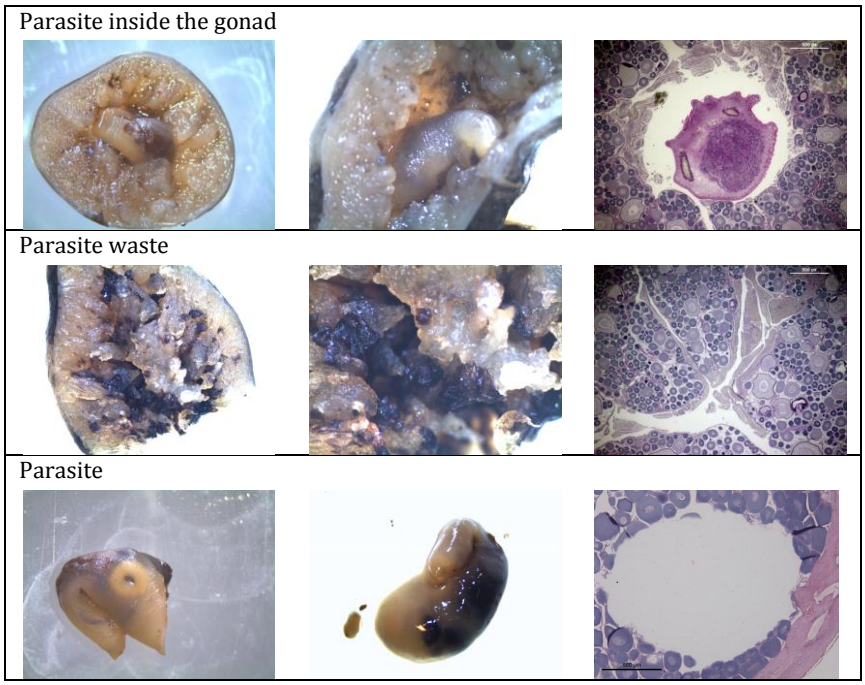

Fig. 1. Damage in gonads caused by parasites. Parasite damages and wastes remain in the gonad. Pictures on the right shows gonadal parasite damage observed under microscope.

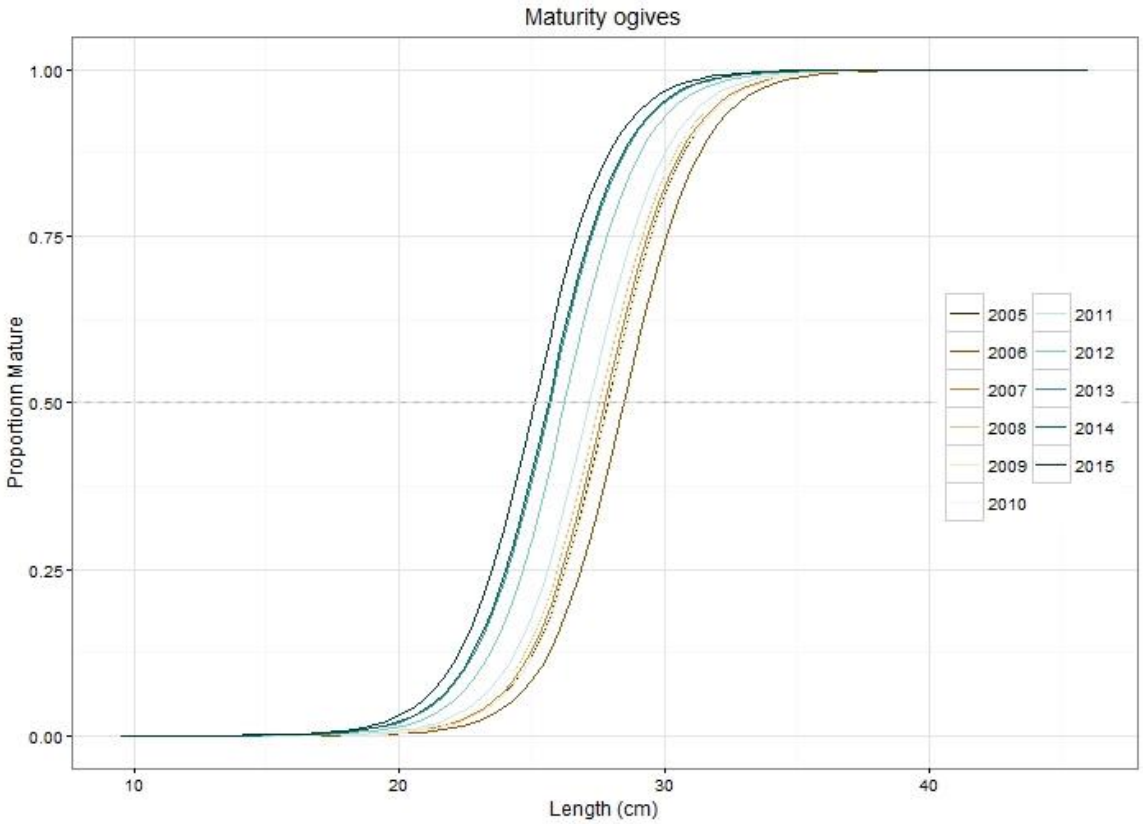

Fig. 2. Maturity ogives for females of roughhead grenadier by year.

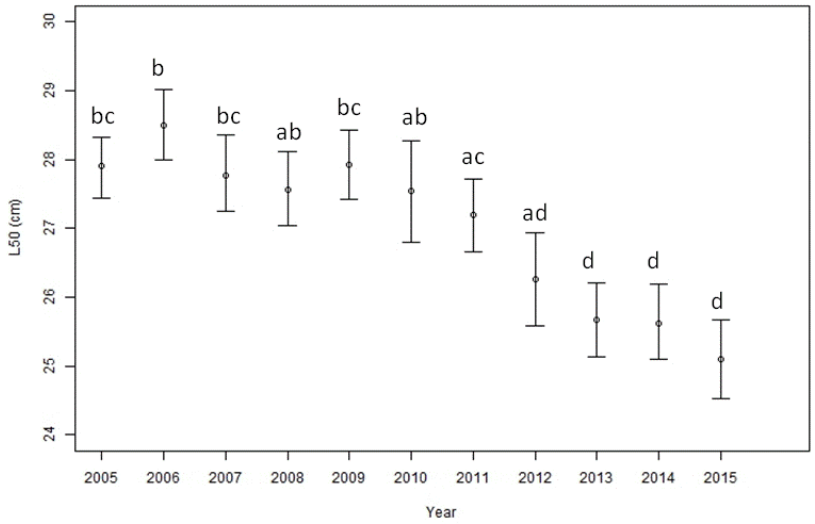

Fig. 3 L_{50} estimation with correspondent bootstrap confidence intervals at 95% by year**.** Between years post hoc differences in maturity ogives are marked with letters. Identical letters denote no significant differences among years.

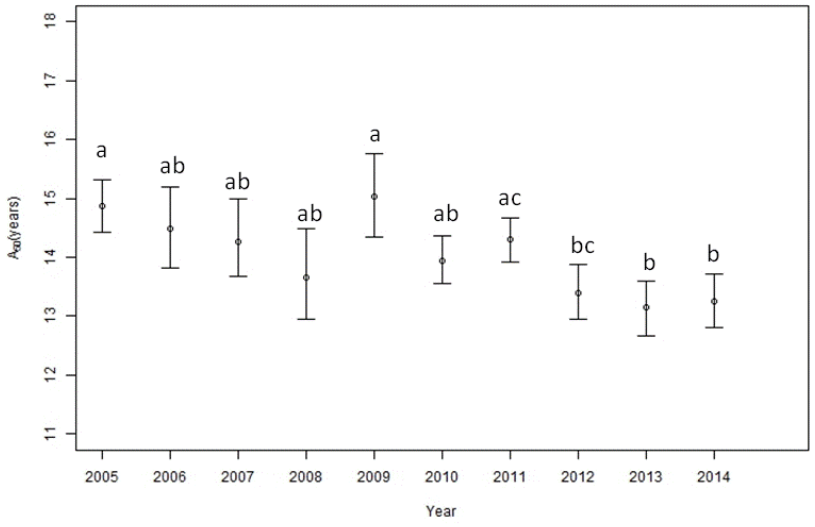

Fig.4. A_{50} estimation with correspondent bootstrap confidence intervals at 95% by year from 2005 to 2014. Letters represent pairwaise differences.

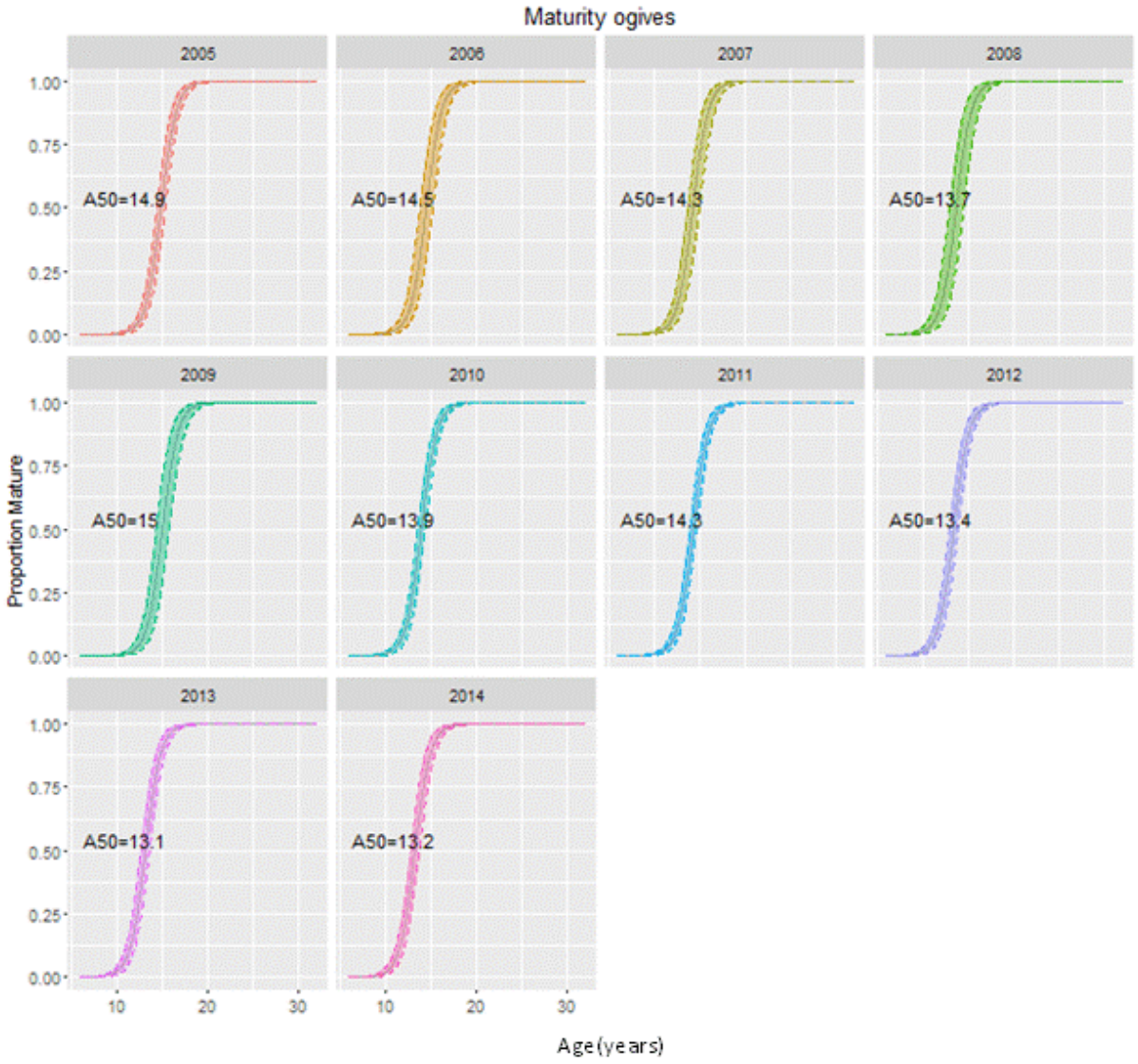

Fig. 5. Maturity ogives for females of roughhead grenadier by year. The 95% confidence limits are shown by dashed lines. A_{50} estimate is also indicated by year.